A PRELIMINARY ACCOUNT OF THE SPERMATOGENESIS OF BATRACHOSEPS ATTENUATUS, POLYMORPHOUS SPERMATOGONIA, AUXOCYTES AND SPERMATOCYTES.

GUSTAV EISEN, PH.D.

INTRODUCTORY.

THIS paper is a preliminary report of my investigations on the spermatogenesis of *Batrachoseps attenuatus*. The memoir will be published in the *Journal of Morphology*, Vol. XVII, No. 1. As, however, some considerable time must elapse before the paper can be published, it has been deemed proper to publish this short extract covering a few of the more important points.

Batrachoseps is adult in the months of June and July, and is at this time difficult to find, as it is then estivating deep below the surface. The testes were fixed by my iridium-chloride method and sectioned in paraffin. The stain used was the iron haematoxylin, according to Benda, and after-staining with congo. The sections were studied with Zeiss Apochromate, 2 mm. Ap. l. 40. The light used was the incandescent gas-light passing through the achromatic filter described lately in the *Zeitschrift f. wiss. Microscopie*, Bd. XIV, p. 444.

The figures illustrating this paper are entirely diagrammatic. No special effort has been made to insert the correct number of chromioles in the chromosomes; and the author's want of skill in preparing this kind of drawing will account for many other discrepancies.

CONSTITUENTS OF THE CELL.

The following general division of the structures of the cells of the testes is proposed: Cytosome, Caryosome, and Archo-

some. This division is in accordance with the one proposed in my paper on the plasmocytes in the blood of *Batrachoseps*.[1]

The cytosome comprises all that part of the cell situated exterior to the nucleus except the archosome. The cytosome contains the following distinct structures: cytoplasm proper, plasmosphere, hyalosphere, granosphere, metaplasmic secretions, cytoplasmic membrane, and cell wall. None of these

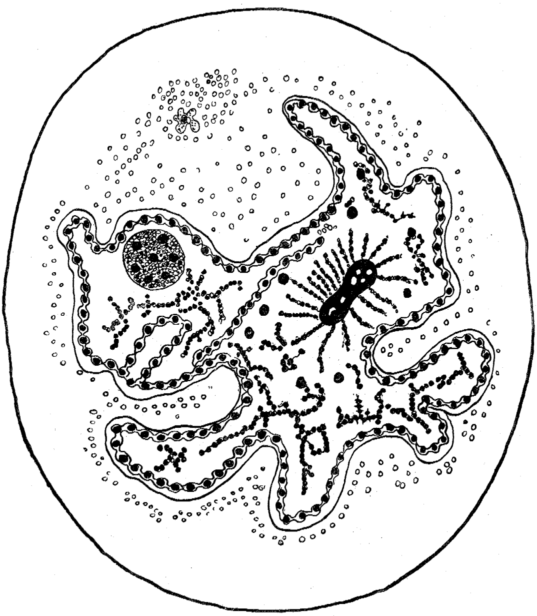

FIG. 1.—A polymorphous spermatogonium in the "perfect resting stage." The form of the nucleus allows the most perfect metabolism. Numerous chromioles are connected by a thread of chromoplasma. A network of linosomes is partially indicated, the individual granules being connected by Linopodia. A large chromoplast with endochromatic granules. Eight parachromatic granules. A single archosome in the cytoplasm, the latter only partially indicated by small open circles. A single large linoplast, with seven endonucleolar granules.

structures are in any way intimately connected with the archosome. The three spheres mentioned above surround each other, like three concentric shells, at the time when the cell is in partial resting stage; but at a later stage, or as soon as the prophase is entered, these spheres break up and scatter in the cytoplasm proper. Each one of the spheres constitutes an independent structure, and they are not developed one from the other.

[1] *Proc. Cal. Acad. Sci.*, 3d Ser., Zoöl. Vol. i, No. 1.

The plasmosphere is the outermost sphere; the granosphere is the innermost one. The plasmosphere is the first one to break up into minor parts. These arrange themselves in the equatorial of the cell and serve as material for the mantle fibers

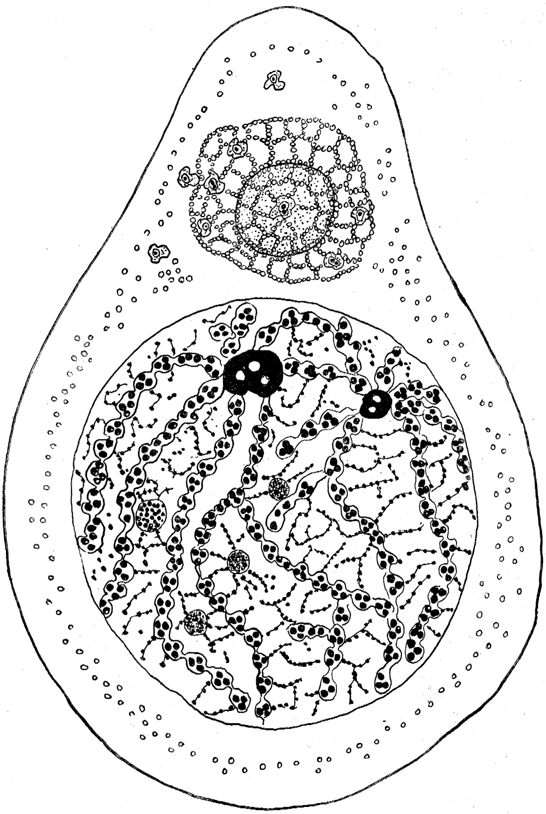

Fig. 2. — Auxocyte in the "imperfect resting stage," showing the formation of leaders consisting of round chromioles surrounded by a film of chromoplasm. The leaders start from two chromoplasts of unequal size, both containing endochromatic granules. The leaders are connected by a linosomic network. Four linoplasts. In the cytoplasm are seen the two spheres, the inner one, the granosphere, containing the archosome. There are eight accessory archosomes, some in the plasmosphere, others in the cytoplasm. The two spheres are of a foam-like structure. The cytoplasm is only partially indicated.

and for the new cell wall which is formed when the two daughter-cells separate.

The granosphere remains longer, but when it breaks up it furnishes material for the fibers of the central spindle. It also constitutes the main dwelling place of the archosome.

The archosome, or centrosomal structures, consists of three distinct parts, situated one interior to the other. These three parts constitute one single organized and individualized body — the archosome. The most interior part is the centriole ; this centriole is surrounded by a thin layer or zone — the somosphere. The somosphere is surrounded by a generally non-staining zone — the centrosphere. There are one or more centrioles.

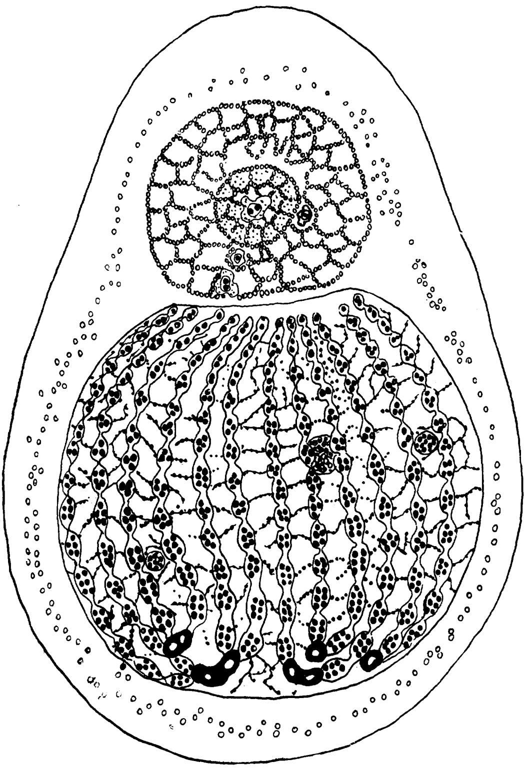

FIG. 3.—An auxocyte in the "bouquet stage." There are twelve leaders starting from five chromoplasts. The leaders consist of chromomeres containing chromioles suspended in a film of chromoplasm. The spheres are of a foam-like structure. There are three accessory archosomes and one archosome with two centrioles. The open space between the inner granosphere and the outer plasmosphere represents the hyalosphere. The cytoplasm is only partially indicated.

Both the somosphere and the centrosphere are amoeboid, especially the centrosphere. The latter constitutes the organ of locomotion of the archosome. There are besides the archosome several accessory archosomes. The function of the

archosome is to conduct the development and evolution of the fibers of the mitotic figures. The function of the accessory archosomes is to conduct the formation of the contractile fibers and perhaps furnish material for their construction. They also conduct the "fiber cones."

The location of the archosome is variable; it is sometimes situated in the granosphere, but is at other times found outside

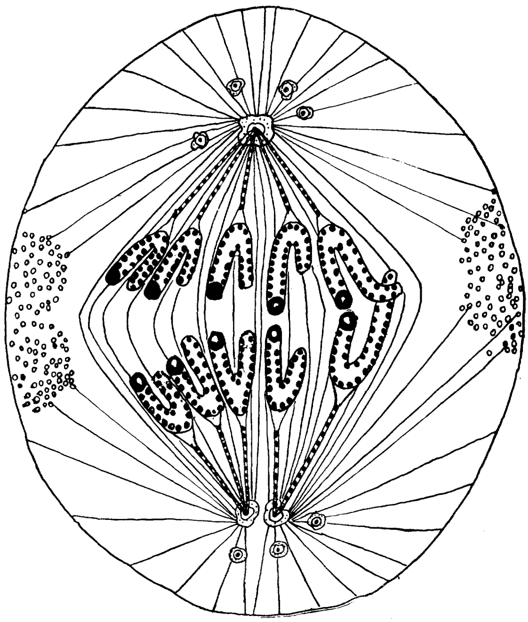

FIG. 4.—An auxocyte in the beginning of the anaphase. Only a few of the chromosomes are indicated. At each pole there are one and two archosomes and three and four accessory archosomes. The chromosomes contain chromioles suspended in chromoplasm. At the apex of each chromosome there is seen a chromoplast with endochromatic granules. To the right and left in the cell are seen agglomerations of plasmosphere indicating the position of the new cell wall, which is to separate the two daughter-cells. The chromosomes are seen to be connected with the chromiole by contractile fibers, the latter consisting of granules enclosed in a common sheath. The spindle fibers as well as the polar fibers start from the centrosphere.

of it. The accessory archosomes are of the same structure as the archosome, and one of the former may assume the function of the latter.

The accessory archosomes, if too numerous, are expelled from the cell, and then become paracellular bodies. Similarly, parts of the spheres are also expelled from the cell.

The nucleus contains the following more or less distinct

parts: chromioles, chromomeres, chromosomes, chromoplasts, linoplasts, linin, chromoplasm, endochromatic granules, and parachromatic granules.

Chromioles. — These are the most minute of the visible organized and individualized primary structures of the nucleus, and are the most important constituents of the chromosomes, probably being the carriers of heredity. They appear as minute globules, staining darker than the other parts of the nucleus except the chromoplasts. The chromioles are of a certain size and number in every species of nucleus and in every perfect chromosome. They are, as a rule, arranged in a regular manner in the chromosomes and in the chromomeres. During the absolute resting stage of the cell the chromioles are situated free in the nucleus, connected only by tiny filaments of linin and chromoplasm; while during the mitotic stages they are grouped into chromomeres, and these again into chromosomes. With absolute resting stage is indicated only absolute rest from "mitotic work." During this stage active metabolism is carried on.

There are thirty-six chromioles in every perfect chromosome, and these are divided among six chromomeres, each chromomere containing six chromioles. The chromioles are surrounded by a connective, apparently homogenous substance — the chromoplasm. The chromoplasm thus constitutes the greatest bulk of the chromosome.

The chromomeres are small aggregations of chromioles from three to six in number, according to the stage of development of the nucleus. The chromosomes in the polymorphous nuclei are twenty-four in number, but in the other testes cells there are only twelve. Each perfect chromosome contains six chromomeres.

In the resting stage of the polymorphous spermatogonia we find in the nucleus one or more large dark-staining bodies — the chromoplasts (net-knots). These chromoplasts are particular and most important organs of the nucleus. Their function is to attract the chromioles and to arrange them first into leaders, and later, through certain changes of the leaders, into chromosomes. The chromoplasts finally divide up into as many parts as there are chromosomes, one part adhering to each chromo-

some through its entire existence. The chromoplasts are characterized by one or more highly refractive endochromatic granules, which probably serve as nourishment for the chromi-

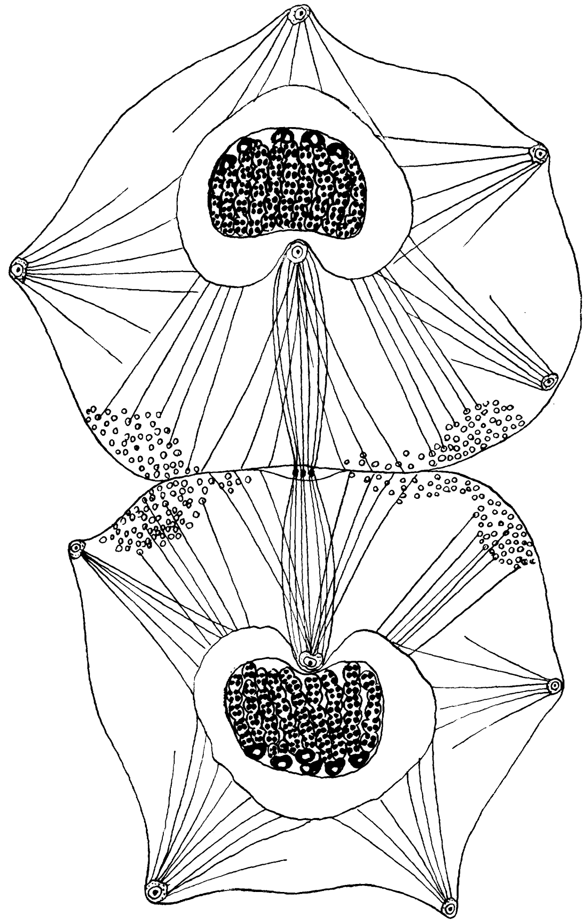

Fig. 5. — Two daughter-cells of an auxocyte connected by a spindle bridge. There are eight accessory archosomes at the apex of as many fiber cones. Two archosomes are connected by a central spindle. In the latter is seen a mid-body consisting of three condensation granules. The chromosomes are being regenerated, and the chromoplasts appear at the angle of the chromosomes instead of at the apex, as in the last cell stage. In one nucleus are seen five, in the other six chromoplasts with endochromatic granules. Between the true nuclear membrane and the false membrane is an open space caused by the false membrane being pulled away by the fiber cones.

oles. The chromoplasts serve as landmarks by which the position of the chromosomes can be ascertained with great accuracy.

The linin consists of minute granules — linosomes — arranged in a more or less regular network, which latter at certain times supports the elements of the chromosomes. The true nucleoli or linoplasts are principally agglomerations of linosomes, and serve as storage reservoirs for the linin network.

The nuclear membrane is formed apparently from linin and not from cytoplasm proper. During the anaphase a false nuclear membrane is formed from cytoplasm proper, but this membrane is again dissolved as soon as the object for which it is formed is accomplished.

SPINDLES AND SPINDLE FIBERS.

The following varieties may be segregated: mantle fibers, polar fibers, central spindle fibers, contractile fibers, retractile fibers, and fiber cones. All these fibers originate only in connection with an archosome. The contractile fibers alone are directly connected with the centriole of the archosome. All the other fibers and rays emanate from the outer margin of the centrosphere of the archosome, but do not penetrate into this sphere, and accordingly are not connected with the centrioles or the somosphere. The material for the mantle fibers is furnished from the granules and the secretions of the plasmosphere; while the material for the central spindle is furnished by the granules and secretions of the granosphere.

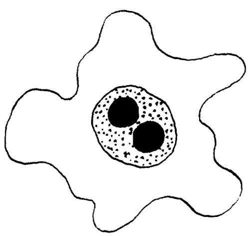

FIG. 6. — An archosome consisting of an outer centrosphere, an inner somosphere, with two centrioles.

The contractile fibers are those which connect the chromosomes with the archosome. They are from the beginning of a different structure from any of the other fibers, being beaded and highly contractile. Their structure strongly recalls that of muscle fiber.

The fiber cones are particular structures, so far not met with in any other cells. They consist of bundles of fibers held together at one point by an accessory archosome, while the distal ends of the fibers are attached to the false nuclear membrane

formed around the nucleus at the time of the anaphase. The archosome moves away and carries with it the fibers, which pull away the false nuclear membrane, thus causing a vacuole to form around the nucleus. The object of all this is probably to enable the nucleus to develop without the interference of surrounding structures. These fiber cones are frequently very numerous, as many as seventeen having been counted in a single cell. They are of large size and cause the cell membrane to be pushed out.[1]

The spindle bridge, which connects two or more cells, consists of the remnant of the central spindle. As the spindle bridge exists only in cells which commence the same phase of mitosis at the same time, it is probable that the purpose of the spindle bridge is to time or regulate the commencement of this mitosis. The mid-body of the spindle bridge serves probably as a storage reservoir for the cytoplasm of the spindle bridge.

Varieties of Cells.

The testes of *Batrachoseps* contain four distinct varieties of cells, as follows: polymorphous spermatogonia, auxocytes, spermatocytes, and spermatids. These originate one from the other in the order mentioned above. Of these varieties there are one or more generations. They are characterized as follows:

Polymorphous Spermatogonia. — These possess a perfect resting stage in which the nucleus is polymorphous as regards form, being greatly indentated and folded during the perfect resting stage. The nucleus during this stage contains neither chromosomes nor chromomeres, the chromioles being scattered about and not connected with the chromoplasts. These cells give rise to several generations of cells of the same nature, with the exception that there is no perfect resting stage like the one in the mother-cell, and that consequently the nucleus is not folded, but perfectly even, round, or oblong. The mitosis of the poly-

[1] Fiber cones of similar appearance, but of a different nature, have been described by botanists from the pollen cells of higher plants. These cones, however, do not possess archosomes.

morphous spermatogonia and their offspring is through twenty-four chromosomes and somatic division. The last generation of these cells gives rise to the auxocytes.

The auxocytes are characterized as follows: They possess a bouquet stage; they are the first cells with twelve chromosomes; their mitosis is heterotypic and by equation; they pos-

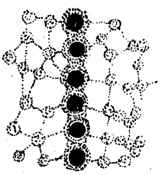

FIG. 7.

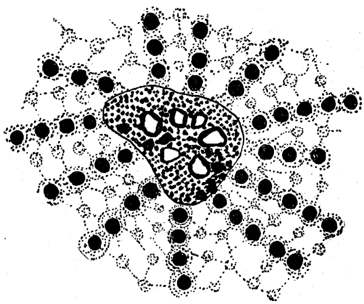

FIG. 8.

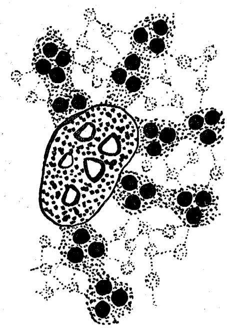

FIG. 9.

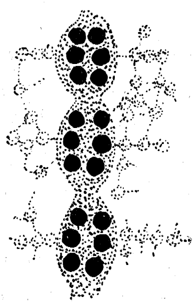

FIG. 10.

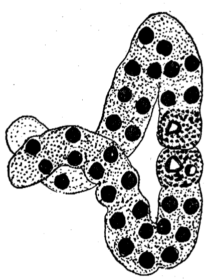

FIG. 11.

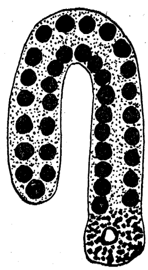

FIG. 12.

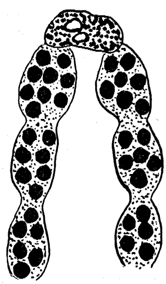

FIG. 13.

FIGS. 7–13 represent a broken series of leaders illustrating the formation of the leader and the chromosome.

FIG. 7.— Isolated row of chromioles surrounded by chromoplasm and suspended in a network of linosomes.

FIG. 8.— Chromoplast with twelve leaders of chromioles. From the imperfect resting stage of the polymorphous spermatogonium.

FIG. 9.— Chromoplast with five leaders. Each leader is made up of chromomeres, and each chromomere consists of three or more chromioles surrounded by chromoplasm. A network of linosomes between the chromomeres.

FIG. 10.— Three chromomeres, each with six chromioles surrounded by a chromoplasm and suspended in a network of linosomes.

FIG. 11.— A pretzel chromosome containing chromioles and two chromoplasts with endochromatic granules.

FIG. 12.— A chromosome from the metaphase. It contains thirty-six chromioles and a terminal chromoplast with an endochromatic granule.

FIG. 13.— Part of a chromosome from the spermatocyte.

sess no perfect resting stage, the chromioles being arranged into leaders and always connected with the chromoplasts; there is but one generation; the daughter-cells are the spermatocytes; and they have numerous fiber cones at the end of the anaphase.

The spermatocytes are characterized as follows: They have numerous fiber cones in the beginning of the mitosis, homotypic mitosis with twelve chromosomes and with equation division; no bouquet stage and no perfect resting stage; but one generation; the daughter-cells are the spermatids which give rise to the spermatozoa through direct development and growth; and the chromosomes are *V*-shaped.

The Mitosis.

The mitosis is the result of two distinct and separate processes which, for the greater part, run parallel and independent of each other, but which meet at certain nodes in order to

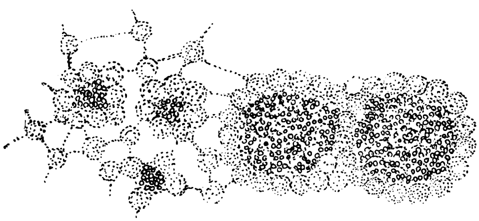

Fig. 14.—A diagrammatic representation of the structure of the granosphere. The dotted globules are cytoplasmic granules, and between them are seen metaplasmic secretions represented by small open rings. The globules are connected by Linopodia, and form a foam structure, partly a network.

accomplish certain objects jointly. These processes are the chromosomic process and the radiosomic process.

The radiosomic process is presided over by the archosome and the accessory archosomes, and consists in the development and evolution of the various fibers and the central spindle, in the evolution of the spheres, and the dissolution of the nuclear membrane. To this process belong also the development and dissolution of the false nuclear membrane and the reabsorption of the fibers.

The chromosomic process is presided over by the chromo-

plasts, and consists in the development and evolution of the leaders out of chromoplasm and the chromioles, the formation of the latter into chromomeres and chromosomes, and the multiplication of the chromioles and their proper distribution in the chromosomes. The two processes coöperate in the separation and equation of the chromosomes, which coöperation commences with the dissolution of the nuclear membrane. To the chromosomic process belongs also the movement of the chromoplasts in the umbrella-shaped and confluent nucleus at the end of the anaphase. With this process the archosomes have nothing to do, as it is accomplished before the nuclear membrane is dissolved by the mantle fibers.

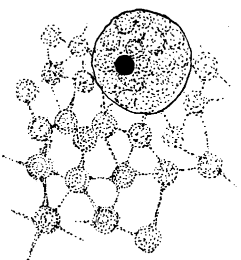

FIG. 15.— A diagrammatic representation of the structure of linosomes and the linoplast. The individual linin granules are connected by means of Linopodia. The linoplast contains linosomes as well as an endonucleolar body.

The radiosomic process commences with the dispersion of the spheres. The plasmosphere is dispersed first, and its granules are arranged in the equatorial of the cell, there to furnish material for the new cell walls. The central spindle fibers are then formed out of material furnished by the granosphere, which is in this way entirely used up. The nuclear membrane is dissolved by the mantle fibers and not by the central spindle fibers. The contractile fibers are formed after the central spindle fibers have reached considerable size.

The chromosomic process begins with the formation of leaders out of chromioles and chromoplasm. The chromioles aggregate into chromomeres, and, later on, a certain number of these form chromosomes. Their formation is shortly as follows: The leaders to the number of twelve are connected with the chromoplasts, and by contraction and a certain arrangement assume the bouquet stage. The leaders then split lengthwise, the two forks being held together by a fragment of the chromoplasts. The chromoplast divides into as many parts as there are to be chromosomes, but each part is always attached to a leader. Next, the two halves of the leader spread apart and twist around each other and thus form a pretzel-shaped chromosome. By this time the nuclear membrane is dissolved and

the pretzel-shaped chromosomes are placed on the central spindle, where they are taken hold of by the contractile fibers, which attach themselves to the prongs halfway between the

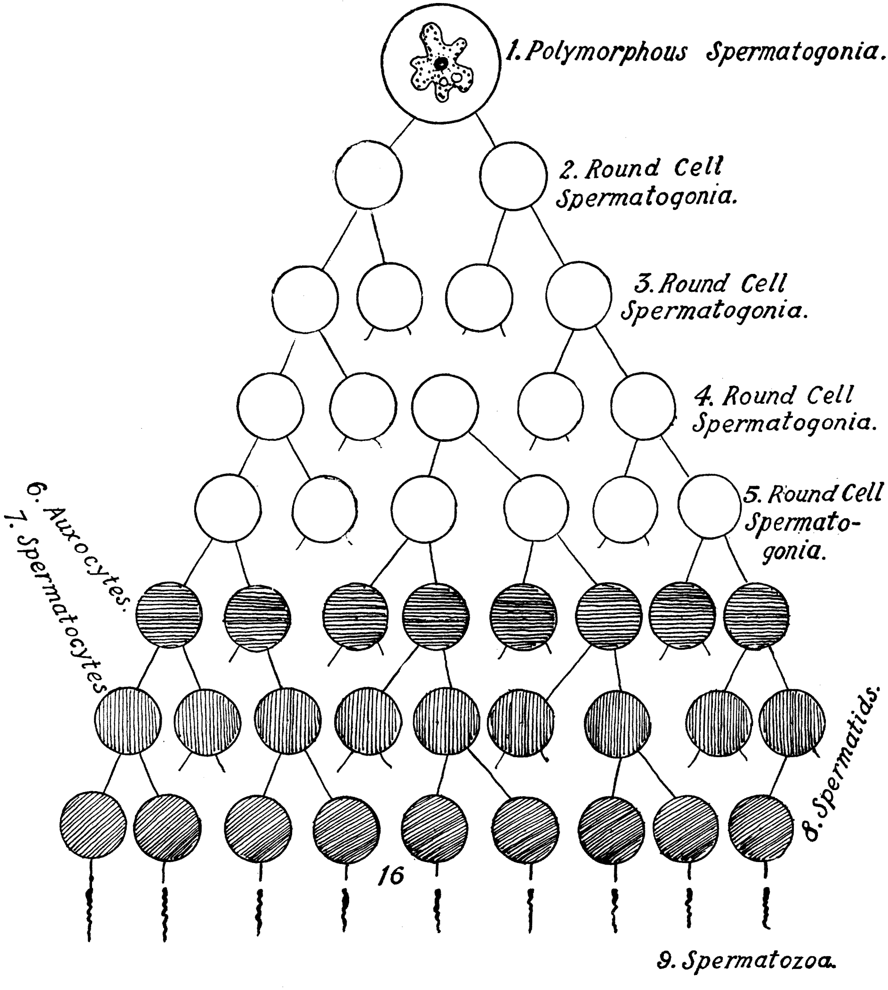

FIG. 16. — Diagram of the cell generations in *Batrachoseps* testes; 1, polymorphous spermatogonia; 2 to 5, four generations of round cell spermatogonia; 6, auxocytes; 7, spermatocytes; 8, spermatids; 9, spermatozoa.

chromoplasts and the free end. Each half is then pulled away and the chromosome is formed by an equation and not by a reduction. In the new chromosome the fragment of chromo-

plast is attached to one of the ends. This process is the one that takes place in the auxocytes.

The next step is the formation of a confluent umbrella stage or ring-like nucleus. The object of this form is to allow the chromoplasts to change their place. When the nucleus is reorganized in the spermatocyte the chromoplasts are found to be situated not at the end of each chromosome, but at the angle of the fork. This change of position could not take place except through the medium of an umbrella-shaped nucleus. During this stage the chromioles are also doubled. The nucleus now passes through a stage of growth which is facilitated through the large vacuole which is formed around the nucleus with the aid of the fiber cones and the accessory archosomes.

In the spermatocyte the central spindle is frequently formed from two opposite fiber cones left over from the last mitosis. The chromosomes of the spermatocytes are *V*-shaped before mitosis. They are divided longitudinally in the way usual in the homotypic mitosis, and by equation, not by reduction. During the prophases of the radiosomic mitosis the superfluous archosomes are expelled from the cell and remain for some time as paracellular bodies between the cells.

PERMANENCY AND NATURE OF THE CELL STRUCTURES.

The cytosome proper contains no permanent structures of any kind. The plasmosphere, hyalosphere, granosphere, the various kinds of fibers, as well as the central spindle, are all ephemeral structures which are developed by rearrangement of preëxisting granula, and which again disperse when their function is over. The granula contained in the cytosome is at least of four different kinds, and everything points to the conclusion that one kind of granula is never converted into any other kind. In other words, the granula of the granosphere is not evolved from the granula of the plasmosphere, etc., but both are independent and individualized primary structures as compared with the secondary ones of spheres and fibers. For the principal granula of the cell the following terminology is proposed : cytosomes, plasmosomes, hyalosomes, somosomes, granosomes, and linosomes, the latter being of nuclear origin.

If we turn to the nucleus, we find similarly that the chromomeres, the chromosomes, and the leaders are also ephemeral and secondary structures which form and disperse, the chromioles alone being the permanent individualities of the chromosomic structures. The nucleus then contains the following permanent granula: linosomes, the chromoplasmic granula, the chromioles, and the granula composing the chromoplasts. The permanent structures of the cell are the centrioles, the chromioles, and the chromoplasts. As regards the primary parts of these last-mentioned structures we are yet in doubt, but there is every reason to believe that these structures are of a highly complicated nature.

Similarly the fibrillar and alveolar structures of the protoplasm are only secondary, ephemeral, and temporary. With proper optical means we see that the alveole, as well as the reticulum, is built up of granules. These granules adhere to each other by means of minute projections or arms, for which I have proposed the name of Linopodia. The ultimate visible structure of the protoplasm is thus a granule, capable of projecting and retracting Linopodia.

For a fuller explanation and demonstration of these facts I must refer to the larger paper now in the hands of the publisher of the *Journal of Morphology*.

Biological Laboratory,
California Academy of Sciences,
San Francisco, California.

ERRATUM.

In No. 1, p. 1, 13th line, last word, read *ganglion* in place of "*gland.*"